Melanocortin 5 receptor plays a major role on adipocyte lipid metabolism

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Introduction

During the last 20 years, a crucial role in the remodelling of body weight has been attributed to melanocortins. They are known to regulate lipid metabolism in adipose tissue by increasing lipolysis rate. However the precise melanocortin receptors involved and the molecular mechanisms driving melanocortin-mediated lipolysis are still unknown and constitute the basis of our work.

Because Melanocortin 5 Receptor (MC5R) deletion in mouse generates defects in sebaceous gland secretion [1] and MC5R stimulation increases fatty acid oxidation in muscle cells [2], these receptor seems to have a role on energy homeostasis and we are particularly interested on the study of its action in adipocytes.

Methods

3T3-L1 differentiation

3T3-L1 pre-adipocytes were diffrentiated by incubation with DMEM containing 10% fetal bovine serum (FBS), 10µg/ml insulin (INS), 250nM dexamethasone and 0,5mM 3-isobutyl-1-methylxanthine during four days. Cells were then allowed to differentiate for seven more days in medium with DMEM, FBS and INS.

siRNA

After differentiation, adipocytes were transfected with 10nM MC5R siRNA (Ambion) using Lipofectamine 2000. Forty-eight hours later, cells were serum-deprived over-night. All assays were performed 72 h after siRNA transfection. A non-targeting siRNA (Sigma) was used as a control.

Real-time PCR

Extraction of total RNA was performed using RNeasy® Plus kit (Qiagen) and cDNA was generated by RevertAid™ H Minus (Fermentas) using 5µg of total RNA. Real-time PCR was carried out with iTaq™ SYBR® Green Supermix (Bio-Rad) using the 7000 Real-Time PCR System (Applied Biosystems).

Western blotting

Cell lysates were prepared from adipocytes after treatment with 1µM alpha-MSH for several time-points. For ERK1/2 inhibition, UO126 was added 30min before alpha-MSH stimulation. Extracted proteins were resolved on 10% SDS-PAGE gels and transferred onto nitrocellulose membranes. Specific primary antibodies were used for detection of ERK1/2 and ACC forms, perilipin and phospho-HSL (Cell Signaling) and α-tubulin (Sigma). A horseradish peroxidase coupled secondary antibody was then visualized by enhanced chemiluminescence (SuperSignal West Pico Chemiluminescent Substrate, Pierce).

Immunofluorescence

After cell fixation in 4% PFA, activated forms HSL were detected by an anti-phospho-HSL antibody (Cell Signaling) and a secondary antibody Alexa®488. Nuclei were stained with DAPI.

Glycerol Release Assay

Fully differentiated adipocytes were treated with 1µM alpha-MSH or isoproterenol for 4h and the incubated medium was collected for glycerol quantification using Free Glycerol Reagent (Sigma).

References

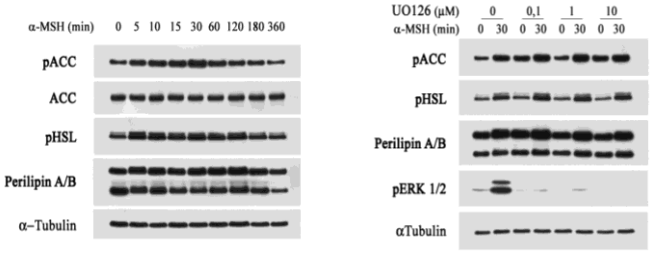
[1] Cone RD (2006) Endocr Rev 27(7):736-49
[2] An et al (2007) J Biol Chem 282(5):2862-70

Acknowledgments

This work was funded by:
Sociedade Portuguesa de Endocrinologia, Diabetes e Metabolismo and ABBOTT;
Tanita Healthy Weight Community Trust
Rodrigues AR was supported by POCI 2010, FSE and "Fundação para a Ciência e Tecnologia" (SFRH/BD/22024/2005).

Results

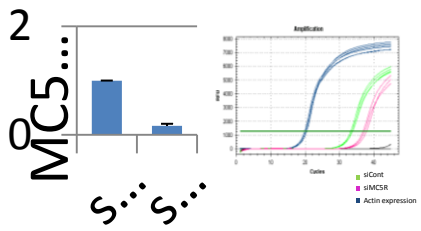
Molecular mechanism of alpha-MSH-mediated lipolysis in 3T3-L1 adipocytes



Alpha-MSH increases phosphorylated levels of Acetyl-CoA Carboxilase (ACC), Hormone Sensitive Lipase (HSL) and Perilipin A/B by an ERK1/2 independent mechanism.

MC5R role on lipid metabolism of 3T3-L1 adipocytes

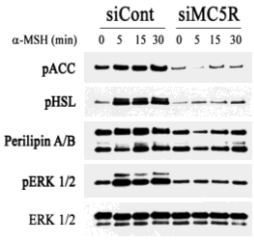
Real-time PCR analysis of MC5R silencing



MC5R expression was efficiently reduced by siRNA

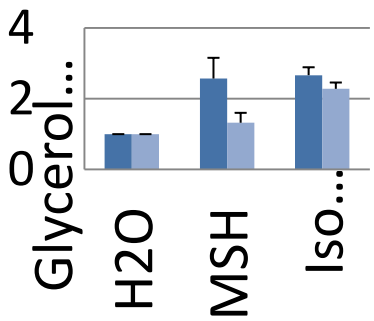
Approximately 80% of MC5R knockdown (siMC5R) was obtained when compared with a non-targeting control siRNA (siCont).

Alpha-MSH signaling in MC5R-depleted adipocytes



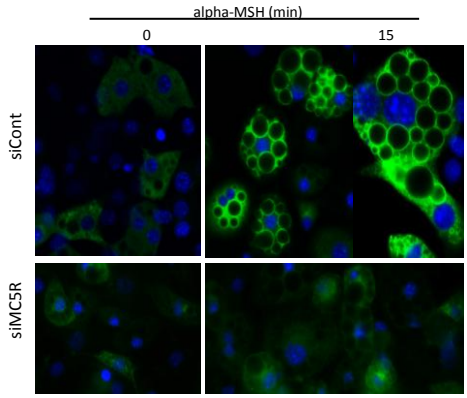
The ability of alpha-MSH to phosphorylate ACC, HSL, Perilipins and ERK1/2 decreases when MC5R expression was reduced by siRNA.

Glycerol Release in MC5R-silenced adipocytes



The ability of alpha-MSH to promote glycerol release was dramatically reduced with MC5R silencing.

Immunofluorescence localization of phospho-HSL



After MC5R siRNA, the activated form of HSL (phospho-HSL) are no longer able to localize on the surface of lipid droplets with alpha-MSH stimuli.

Conclusions

- MC5R has an active role on the regulation of adipocyte lipid metabolism.
- HSL and Perilipins are regulators of MC5R-mediated lipid hydrolysis.